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Treatment with 2-Methoxyestradiol

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13. ABSTRACT (Maximum 200 Words) <p>2-ME₂ is an endogenous estrogen metabolite that inhibits the proliferation of breast and other human cancer cell lines. 2-ME₂ also has potent anti-angiogenic and anti-tubulin properties, and it may inhibit estrogen-induced carcinogenesis in the mammary gland. We set out to test the hypothesis that 2-ME₂ might be a substrate for sulfate conjugation and, therefore, that individual variations in the sulfation of 2-ME₂ might contribute to individual differences in its metabolism, pharmacokinetics and therapeutic efficacy. As a first step, we tested 2-ME₂ as a substrate for 7 human sulfotransferase (SULT) isoforms -- as well as all of the common allozymes for SULT1A1 and 1A2. Substrate kinetic studies were conducted in two stages -- starting with concentrations over 5 orders of magnitude, followed by determination of K_m values over a narrow concentration range. 2-ME₂ was a sulfate acceptor substrate for SULT1A1*1, *2, *3; 1A2*1, *2, *3; 1A3; 1E1; 2A1; 2B1a and 2B1b, with apparent K_m values of 2.5, 5.2, 1.6; 4.2, 111, 5.3; 91; 0.067; 8.3; 4.1 and 4.1 μM, respectively. These results suggest that individual pharmacogenetic variation in sulfate conjugation might contribute to individual differences in 2-ME₂ pharmacokinetics and therapeutic effect.</p>				
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INTRODUCTION

The risk of estrogen-induced breast cancer is affected by the balance between activities of several enzymes involved in the biotransformation of estrogen and its metabolites. 2-methoxyestradiol (2-ME), an endogenous estrogen metabolite that inhibits the proliferation of many human cell lines in vitro and in vivo, is being developed for clinical testing as an anticancer agent. 2-ME has unique biological properties not shared with the other estrogen metabolites. It has potent anti-angiogenic activity in vitro and in vivo as well as antitubulin properties. While the exact mechanism of antiproliferative activity of 2-ME is unknown, emerging evidence suggests that 2-ME may inhibit estrogen-induced carcinogenesis in target tissues such as the mammary gland. Conjugation of 2-ME, catalyzed by sulfotransferase (SULT) enzymes may alter its anti-tumorigenic effects in the treatment of breast cancer. Many human SULTs are genetically polymorphic therefore, individual variations in SULT enzyme activity imply variations in the inactivation of 2-ME, with subsequent variations in antitumorigenic activity. We therefore hypothesized that SULT enzyme activities may play an active and important role in the response of breast cancer patients to treatment with 2-ME, through changes in catabolism. Hence, sulfation, may play a role in the therapeutic response of individuals to the treatment of breast cancer with 2-ME.

BODY

The main task was to characterize the SULT enzymes involved in the catabolism of 2-ME₂. This task was completed.

To initiate this work, cDNAs for human *SULT1A1*1*, *SULT1A1*2*, *SULT1A1*3*; *SULT1A2*1*, *SULT1A2*2*, *SULT1A2*3*; *SULT1A3*; *SULT1B1*; *SULT1C1*; *SULT1E1*, *SULT2A1*, *SULT2B1a*; *SULT2B1b* and *SULT4A1* were each ligated into either the eukaryotic expression vector pCR3.1 or p91023B. Sequences of the cDNA inserts were confirmed by DNA sequencing prior to transfection of COS-1 cells using the DEAE-dextran or the Transfast method. Cytosol from transfected COS-1 cells served as a source of recombinant protein.

The resulting recombinant SULT proteins were used for the biochemical characterization of 2-ME₂. Substrate kinetic studies for the sulfation of 2-ME₂ was performed using the modified assay method of Foldes and Meek for sulfotransferases. Because of profound substrate inhibition displayed by SULTs, two sets of experiments were performed for each enzyme. The K_m and V_{max} values were then calculated using the method by Cleland. See appendix.

The long term goal of this project would be to identify the existence of functionally significant polymorphism(s) in the SULT isoform(s) responsible for catabolism of 2-ME₂ in the target tissue. Genotyping of patients prior to treatment with 2-ME₂ would be expected to predict response and/or toxicity, and allow for the tailoring of drug doses to individual patients.

In the appendix are :

Figure 1: Scheme showing the sulfate conjugation of 2-ME₂.

Figure 2: Substrate curves and double inverse plot for 2-ME₂ catalyzed by SULT1E1.

Table 1 : Substrate Kinetic results obtained from this study.

KEY RESEARCH ACCOMPLISHMENTS

The proposed task/work indicated in the concept was completed.

A poster with this work was presented at the 102nd Annual meeting of the American Society for Clinical Pharmacology and Therapeutics (ASCPT), in March 2001, at Orlando, FL.

REPORTABLE OUTCOMES

Adjei, A.A., Wood, T.C. and Weinshilboum, R.M. (2001). 2-Methoxyestradiol (2-ME₂) Sulfation: Possible metabolic pathway. *Clinical Pharmacology and Therapeutics* 69:75. The abstract is attached in the appendix.

CONCLUSIONS

- 2-ME₂ is an endogenous estrogen metabolite formed *in vivo* by the O-methylation of 2-hydroxyestradiol, a reaction catalyzed by COMT.
- 2-ME₂ is being tested as an antineoplastic agent because of its anti-proliferative, anti-angiogenic and anti-tubulin properties.
- Sulfate conjugation is one potential metabolic pathway for 2-ME₂.
- We found that seven of the ten known human SULT isoforms can catalyze the sulfation of 2-ME₂.
- Of the isoforms studied, SULT1E1 had the lowest apparent K_m value for 2-ME₂.
- The common allozymes for SULT1A1 also catalyzed the sulfation of 2-ME₂. Therefore, if this isoform contributes significantly to 2-ME₂ biotransformation *in vivo*, genetic variation in SULT1A1 might contribute to individual differences in 2-ME₂ metabolism, pharmacokinetics and therapeutic efficacy.
- The next step in these studies will require a determination of the relative importance of sulfate conjugation in the metabolism of 2-ME₂ when this agent is administered in a clinical setting.

"SO WHAT"

As a result of these studies, we have evidence that SULTs metabolize the anti-tumorigenic drug, 2-ME₂. Since many of the human SULTs are genetically polymorphic, genotyping patients prior to treatment, perhaps, may predict response and/or toxicity and allow for tailoring of drug doses to individual patients.

REFERENCES

Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254, 1976.

Bradlow, H.L., Miichnovicz, J.J., Telang, N.T., and Osborne, M.P. Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. *Carcinogenesis* 12:1571-1574, 1991.

Campbell, N.R.C., Van Loon, J.A. and Weinshilboum, R.M. Human liver phenol sulfotransferase: assay conditions, biochemical properties and partial purification of isozymes of the thermostable form. *Biochem. Pharmacol.* 36:1435-1446, 1987.

Cleland, W.W. Computer programmes for processing enzyme kinetic data. *Nature (Lond.)* 198:463-465, 1963.

Foldes, A. and Meek, J.L. Rat brain phenolsulfotransferase - partial purification and some properties. *Biochim. Biophys. Acta* 327:365-374, 1973.

Cushman, M., He, H.-M., Katzenellenbogen, J.A., Lin, C.M. and Hamel, E. Synthesis, antitubulin and antimitotic activity, and cytotoxicity of analogs of 2-methoxyestradiol, an endogenous mammalian metabolite of estradiol that inhibits tubulin polymerization by binding to the colchicine binding site. *J. Med. Chem.* 38:2041-2049, 1995.

Cushman, M., He, H.-M., Katzenellenbogen, J.A., Varma, R.K. and Hamel, E., Lin, C.M., Ram, S. and Sachdeva, Y. P. Synthesis of analogs of 2-methoxyestradiol with enhanced inhibitory effects on tubulin polymerization and cancer cell growth. *J. Med. Chem.* 40:2323- 2334, 1997.

D'Amato, R.J., Lin, C.M., Flynn, E., Folkman, J. and Hamel, E. 2-Methoxyestradiol, an endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site. *Proc. Natl Acad. Sci. USA*, 91:3964 -3968, 1994.

Fotsis, T., Zhang, Y., Pepper, M.S., Adlercreutz, H., Montesano, R., Nawroth, P.P. and Schweigerer, L. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. *Nature* 368:237-239, 1994.

Hernández, J.S., Watson, R.W.G., Wood, T.C. and Weinshilboum, R.M. Sulfation of estrone and 17 β -estradiol in human liver: catalysis by thermostable phenol sulfotransferase and by dehydroepiandrosterone sulfotransferase. *Drug Met. Dispos.* 20:413-422, 1992.

Klauber, N., Parangi, S., Flynn, E., Hamel, E. and D'Amato, R.J. Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. *Cancer Res.* 57:81-86, 1997.

Lottering, M.-L., Haag, M. and Seegers, J.C. Effects of 17 β -estradiol metabolites on cell cycle events in MCF-7 cells. *Cancer Res.* 52:5926 -5932, 1992.

Osborne, M.P., Telang, N.T., Kaur, S. and Bradlow, H.L. Influence of chemopreventive agents on estradiol metabolism and mammary preneoplasia in the C3H mouse. *Steroids* 55:114-119, 1990.

Raftogianis, R.B., Wood, T.C., Otterness, D.M., Van Loon, J.A. and Weinshilboum, R.M. Phenol sulfotransferase pharmacogenetics in humans: association of common *SULT1A1* alleles with TS PST phenotype. *Biochem. Biophys. Res. Commun.* 239:298-304, 1997.

Raftogianis, R.B., Wood, T.C. and Weinshilboum, R.M. Human phenol sulfotransferases *SULT1A2* and *SULT1A1*: genetic polymorphisms, allozyme properties and human liver genotype-phenotype correlations. *Biochem. Pharmacol.* 58:605-610, 1999.

Wilkinson, G.N. Statistical estimations in enzyme kinetics. *Biochem. J.* 80:324-332, 1961.

Yager, J.D. and Liehr, J.G. Molecular mechanisms of estrogen carcinogenesis. *Ann. Rev. Pharmacol. Toxicol.* 36:203-232, 1996.

Zhu, B.T. and Liehr, J.G. Quercetin increases the severity of estradiol-induced tumorigenesis in hamster kidney. *Toxicol. Appl. Pharmacol.* 125:149-158, 1994.

Zhu, B.T. and Liehr, J.G. Inhibition of catechol-O-methyltransferase-catalyzed O-methylation of 2 and 4-hydroxyestradiol by quercetin: possible role in estradiol-induced tumorigenesis. *J. Biol. Chem.* 271:1357-1363, 1996.

Zhu, B.T. and Conney, A.H. Is 2-methoxyestradiol an endogenous estrogen metabolite that inhibits mammary carcinogenesis? *Cancer Res.* 58:2269-2277, 1998.

Zheng, W., Xie, D.W., Cerhan, J.R., Sellers, T.A., Wen, W.Q. and Folsom, A.R. Sulfotransferase 1A1 (*SULT1A1*) polymorphism, endogenous estrogen exposure, well-done meat intake, and breast cancer risk. *Cancer Epi, Biomarker Prevent.* In press, 2001.

APPENDICES

Appendix I :

2-METHOXYESTRADIOL (2-ME₂) SULFATION: POSSIBLE METABOLIC PATHWAY. A.A. Adjei, PhD*, T.C. Wood, B.A.* and R.M. Weinshilboum, MD. Mayo Clinic-Mayo Foundation, Rochester, MN

2-ME₂ is an endogenous estrogen metabolite that inhibits the proliferation of breast and other human cancer cell lines. 2-ME₂ also has potent anti-angiogenic and anti-tubulin properties, and it may inhibit estrogen-induced carcinogenesis in the mammary gland. We set out to test the hypothesis that 2-ME₂ might be a substrate for sulfate conjugation and, therefore, that individual variations in the sulfation of 2-ME₂ might contribute to individual differences in its metabolism, pharmacokinetics and therapeutic efficacy. As a first step, we tested 2-ME₂ as a substrate for 7 human sulfotransferase (SULT) isoforms -- as well as all of the common allozymes for SULT1A1 and 1A2. Substrate kinetic studies were conducted in two stages -- starting with concentrations over 5 orders of magnitude, followed by determination of K_m values over a narrow concentration range. 2-ME₂ was a sulfate acceptor substrate for SULT1A1*1, *2, *3; 1A2*1, *2, *3; 1A3; 1E1; 2A1; 2B1a and 2B1b, with apparent K_m values of 2.5, 5.2, 1.6; 4.2, 111, 5.3; 91; 0.067; 8.3; 4.1 and 4.1 μM, respectively. These results suggest that individual pharmacogenetic variation in sulfate conjugation might contribute to individual differences in 2-ME₂ pharmacokinetics and therapeutic effect.

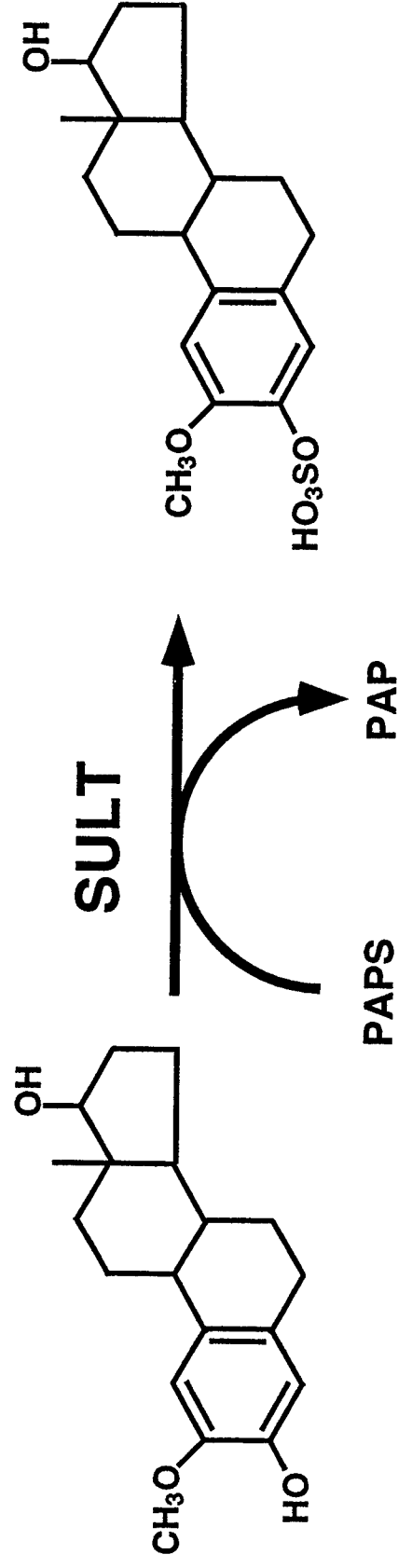
[Supported by DAMD Grant DAMD17-00-1-0684]

Appendix II : See attached Figures and Table on pages 9-11.

N.B.

FIGURES AND TABLE IN APPENDIX II ARE PROPRIETARY DATA.

SULT Catalyzed 2-Methoxyestradiol Sulfation



2-Methoxyestradiol

2-Methoxyestradiol-3-O-Sulfate

Sulfation of 2-Methoxyestradiol by SULT1E1

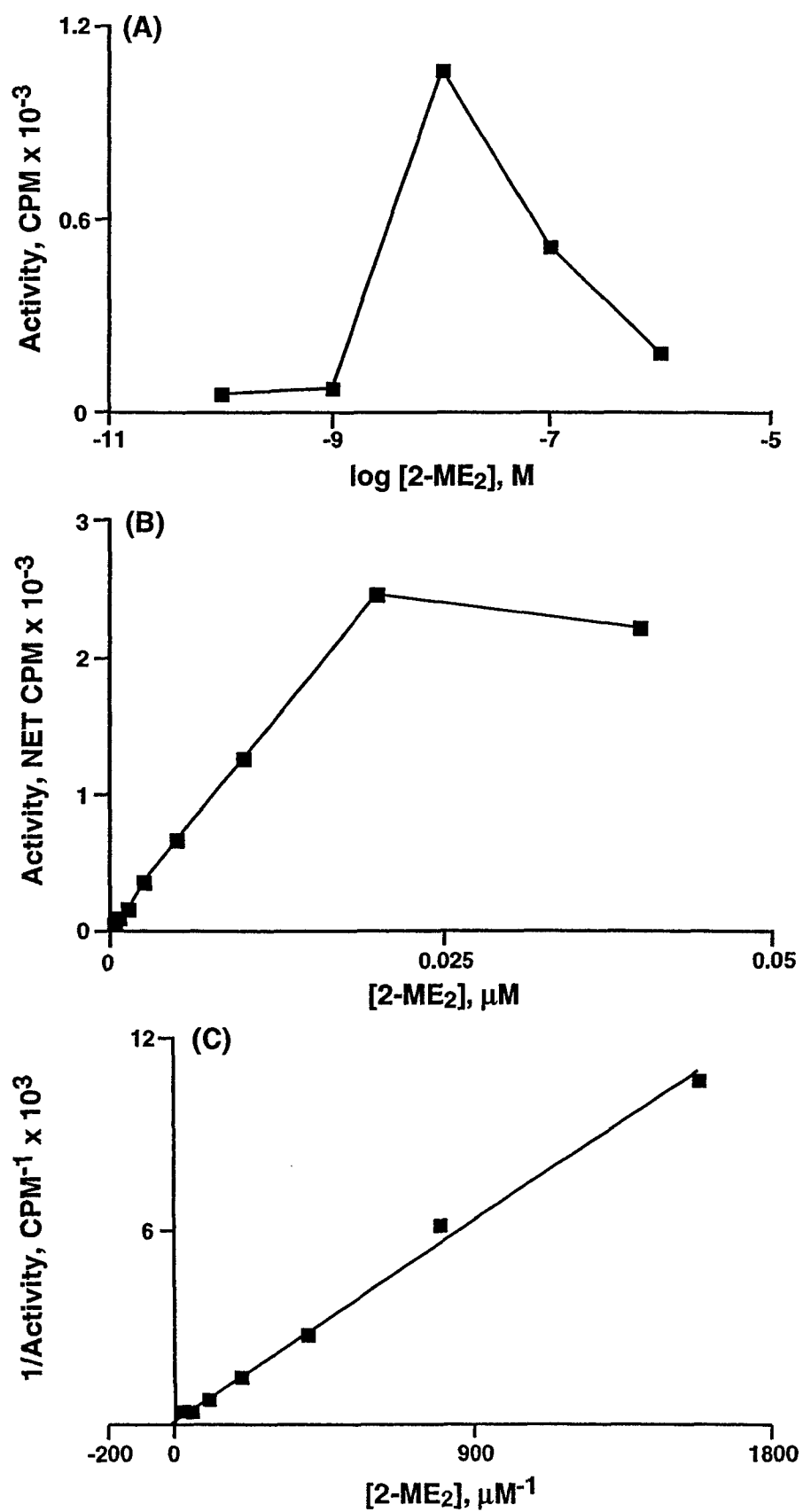


Table 1.

SUBSTRATE KINETICS FOR SULT ISOFORMS :
REACTION WITH 2-METHOXYESTRADIOL

Recombinant SULT Isoforms	Apparent Km Value ($\mu\text{M} \pm \text{S.E}$)	Vmax Units/B-Gal units	V/K($\times 10^3$)
1A1*1	2.5 ± 0.1	2.97	1188
1A1*2	5.2 ± 0.4	3.79	729
1A1*3	1.6 ± 0.2	1.13	707
1A2*1	4.2 ± 0.3	1.56	372
1A2*2	111 ± 0.5	1.51	14
1A2*3	5.3 ± 0.3	0.34	65
1A3	91.4 ± 23.0	0.97	11
1B1	ND	ND	ND
1C1	ND	ND	ND
1E1	0.067 ± 0.0	1.65	24591
2A1	8.3 ± 0.9	0.21	25
2B1a	4.1 ± 0.1	0.51	124
2B1b	4.1 ± 0.3	0.71	173
4A1	ND	ND	ND

ND : No detectable signal



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